U S Air Force Ofc of Scientific Research

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Objectives: The two main objectives of our studies are:

(1) The mechanism in which physical environment, particularly of oxygen tension, influences the gastrointestinal absorption and the metabolism of trace minerals such as iron and zinc.

(2) The evaluation of the effects of dietary deprivation of zinc.

Results: Some pertinent findings are now reported below:

(1) The secondary effect of environment on the gastrointestinal absorption of iron. In case of anorexia, the food intake of adult rats is reduced and if this condition is allowed to exist for several weeks the protein reserve of the animals could become depleted as evidenced by:

(a) the loss of body weight,

(b) and a lowering of plasma protein concentration. It is, therefore, of interest to ascertain whether a mild protein depletion under the circumstance of reduced atmospheric pressure can effect the absorption of vitamin B_{12}. Cyanocobalamine was chosen because of its important physiologic function and its ease of identification and measurement. The data given in Table I show that placing animals under reduced pressure is accompanied by body weight loss and probably protein depletion. The absorption of vitamin B_{12} by such animals was decreased. Since the absorption of vitamin B_{12} depends on the presence of intrinsic factor, a protein containing molecule, it seems reasonable to assume that depletion of protein reserve may cause a reduction of gastric secretion of this compound. This belief is supported by the fact that administration of vitamin B_{12} together with exogenous intrinsic factor isolated from other normal rats enhanced the absorption of vitamin B_{12}.

(2) The effect of housing pregnant rats in reduced atmospheric pressure on the offspring. The lack of sufficient oxygen supply to the pregnant rats may result in damages in the development of the offspring. To this end we have used two groups of pregnant rats (six each) at the 16th day of pregnancy. The day of pregnancy was established by the observation of sperm in the vagina; this date is taken as the first day of pregnancy. During the entire experimental period both groups were offered Purina Laboratory Chow ad libitum. Group A was housed in an enclosed round oil drum (27" long and 14 1/2" diameter) with proper inlet for desired gas mixture and outlet for attachment to vacuum and for mounting of a pressure gauge. The atmospheric pressure for this group of animals was kept at 1/2 atmosphere. The other Group B was kept in a similar drum maintained at 1 atmospheric pressure. The birthweight of the pups as well as the growth rates are

tabulated in Table II. The data demonstrate that the pups born to mothers kept at 1/2 atmospheric pressure were smaller and suffered from growth stunting since they remained smaller over a period of one year, even though they were offered the Purina Chow diet on unlimited amounts under normal conditions, than pups born to mothers kept at 1 atmosphere.

The same mothers in Groups A and B were mated three months later with their respective males. After establishment of pregnancy, the Group A which was confined to 1/2 atmosphere was then kept at 1 atmosphere and the mothers in Group B which were kept at 1 atmosphere during the previous pregnancy now was kept at 1/2 atmospheric pressure. The body weight of the pups at birth and at 12 months of age are also given in Table II. The data demonstrate that animals placed at 1/2 atmospheric pressure produced smaller rats at birth and they suffered growth stunting. This phenomenon may be due to reduction of food intake of the mother during pregnancy, since our other studies suggest that such reduction of dietary intake by as little as 25% of the consumed by control rats on unlimited ration during the last 1/2 of gestation period may conceivably bring about growth stunting of the progeny. The following experiment was, therefore, conducted in which the dietary intake of the mother (Group B) kept at 1/2 atmosphere, but with normal partial pressure of oxygen was pair-fed to those kept at 1 atmosphere (Group C). The results given in the following Table III suggest that placing pregnant rats in reduced atmospheric pressure, but with normal oxygen pressure did not seem to cast young suffering from growth stunting (compare Groups A and C). This experiment will be repeated.

(3) Zn deficiency in rats. One of the objectives of our original proposal was to evaluate the effect of dietary deprivation of zinc. We can now state that we have successfully produced zinc deficient rats rapidly by dietary deprivation by using a diet which contains high quality protein, but low in zinc content. A salt mixture with all trace mineral except Zn was also supplied in the diet. Adequate supplies of vitamins were in the diet. Zinc deficiency in our rats was established by the determination of the total zinc content in certain tissues and by the uptake of radioactive Zn^{65} given subcutaneous by trace amounts. The results tabulated in Tables IV and V show that deprivation of zinc brought about a reduction of total zinc content in tissues and an increase in the uptake of this radioactive Zn in liver, kidneys, pancreas and lungs, after injection of a tracer dose of this radioactive Zn. Physical characteristics of the zinc deficient rats are: graying and loss of hair; failure to gain body weight at the rate comparable to the controls; hypogonadism; dwarfism and atrophy of the testes and seminal vesicles; and hematological abnormalities. All these abnormalities show up in 4-5 weeks of dietary deprivation. The electrophoretic pattern of plasma of Zn deficient animals was normal but the Zn deprived rats appeared to have macrocytic anemia, which can be fully corrected if zinc together with the following vitamin mixtures was given: B_{12}, B_6 and folic acid.

Table I

Effect of Protein Depletion on Co^{60} B_{12} Absorption

Group	Body Weight Loss	Vitamin B_{12}	Intrinsic Factor	Amounts Absorbed (ugm)
Protein Depleted (7)	1/3	+	-	0.033
Not Protein Depleted (6)	0	+	-	0.049
Protein Depleted (5)	1/3	+	+	0.044
Not Protein Depleted (5)	0	+	+	0.044

Mean $\pm$ Standard Error

Oral Dose -.2ugm $Co^{60}B_{12}$ (.2ugm/.26uc)

Gastric Juice Supplement - 2ml

Number in parenthesis - animals per group

Absorption was measured by means of the Fecal Excretion Test.

Table II

Effect of Environmental Atmospheric Pressure on the Growth of Offspring

Cycle	Atmospheric Pressure (atmosphere)	Average Body Weight (gm) At Birth	52 Weeks Male	Female
Group A				
1	1/2	4.1±0.4	325	285
2	1	5.2±0.2	450	368
Group B				
1	1	5.6±0.2	410	330
2	1/2	4.4±0.3	318	295

Table III

Effect of Oxygen Partial Pressure on the Growth of Offspring

Group	Atmospheric Pressure (atmosphere)	Oxygen Partial Pressure (%)	Average Body Weight At Birth	Average Body Weight 52 Weeks Male	Average Body Weight 52 Weeks Female
A	1/2	4	4.2±0.3	350	293
B	1/2	8	5.2±0.2	410	322
C	1	8	5.6±0.3	426	336

Table IV

Zinc Content of Tissues After Dietary Deprivation of Zinc

Treatment	Liver	Kidneys	Pancreas	Lung	Plasma (ugm/ml)
	ugm/gm Wet Tissue				
Control	27.8 ± 0.8[1]	27.9 ± 2.1	24.6 ± 2.0	62.1 ± 2.5	2.10 ± 0.001
Zn Deficient	19.1 ± 1.2	17.6 ± 1.4	13.6 ± 1.0	23.3 ± 1.4	0.49 ± 0.001

[1] SE of Mean

There were six animals in each group.

They were on the experimental diets for six weeks after weaning.

Table V

Uptake of $Zinc^{65}$ in Tissues of Zinc Deprived Animals

Treatment	Liver	Kidneys	Pancreas	Lung	Plasma	Urine	Feces
	% Injected Dose (ID)/gm Wet Tissue				% ID/100 ml	% ID	
Control	2.6 ± 0.2[1]	3.2 ± 0.1	2.4 ± 0.2	1.7 ± 0.2	9.5 ± 2.0	0.76 ± 0.08	1.86 ± 0.4
Zn Deficient	5.8 ± 1.0	4.8 ± 0.4	3.1 ± 0.1	4.7 ± 0.3	4.1 ± 1.6	0.46 ± 0.05	0.58 ± 0.2

[1] SE of mean

There were six animals in each group.

They were given the experimental diets for 8 weeks.